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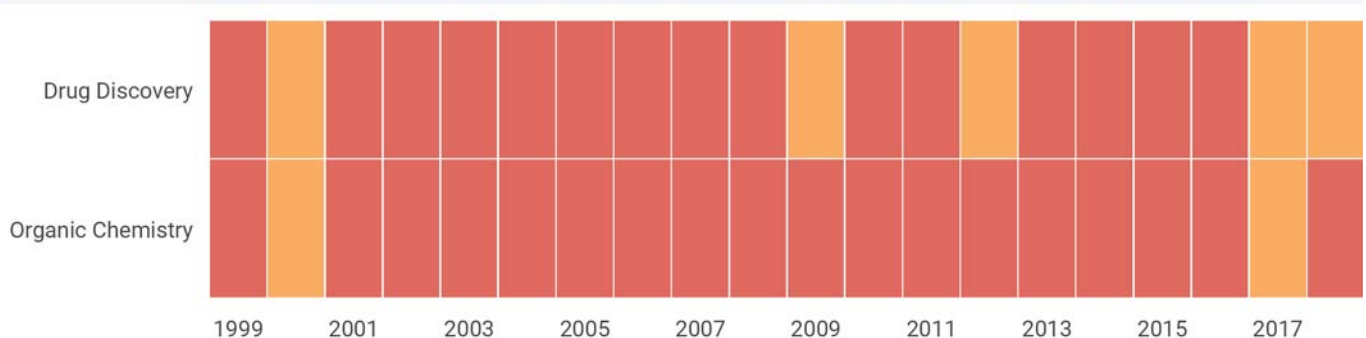
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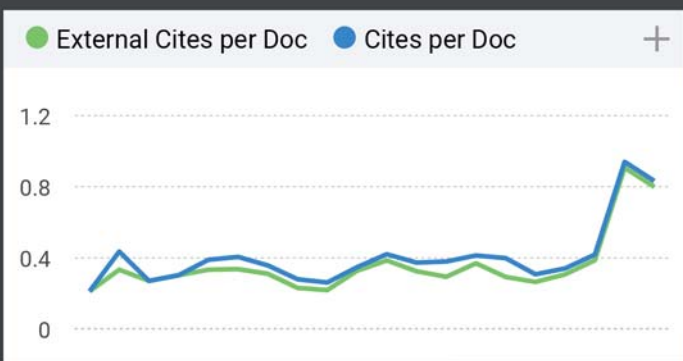
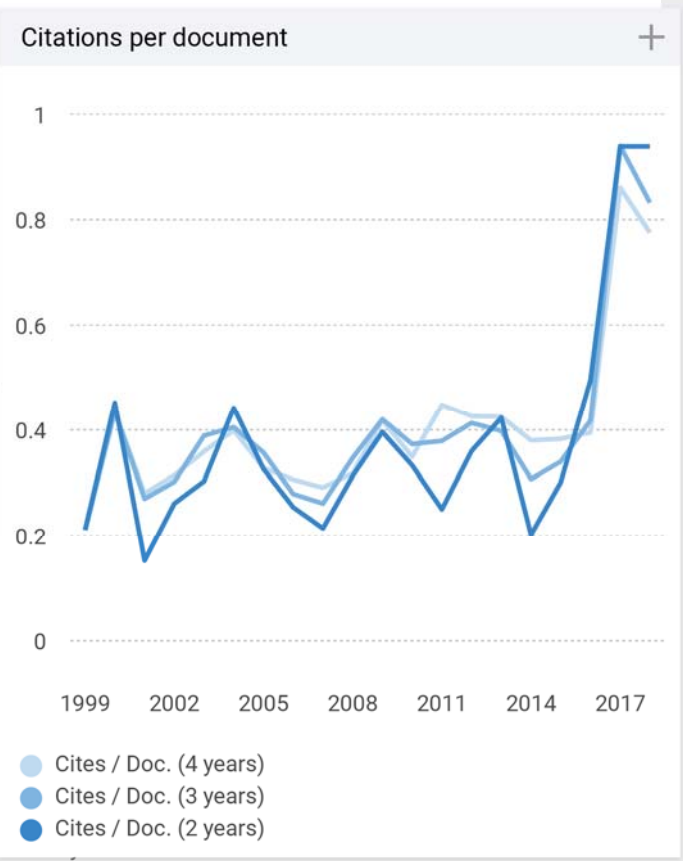


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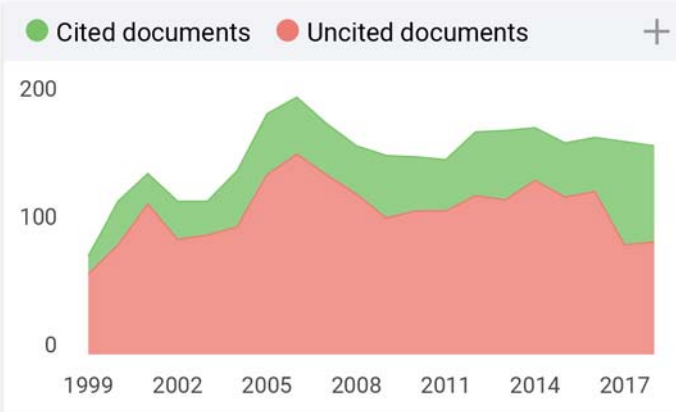
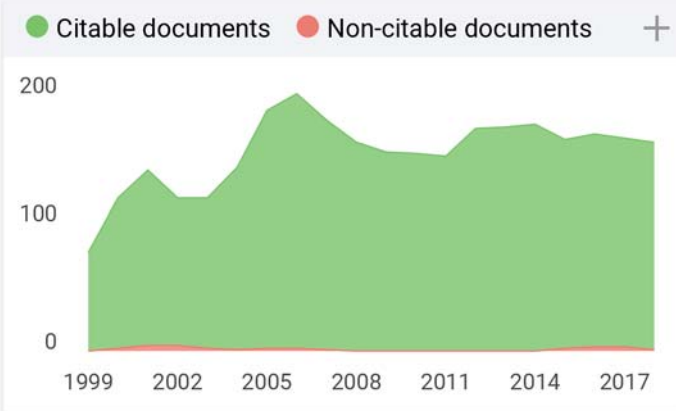
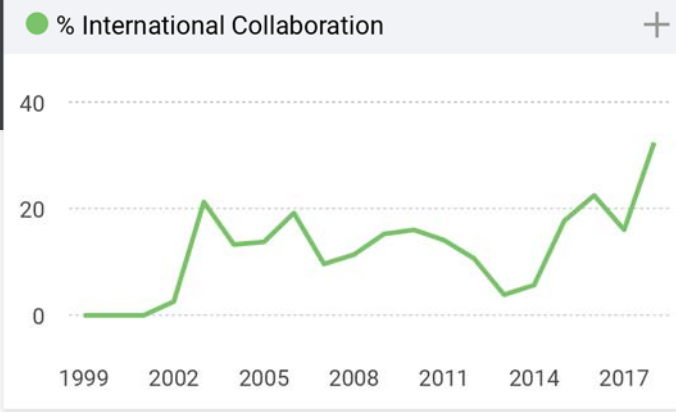
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## Acronyculatin P, A New Isoprenylated Acetophenone from the Stem Bark of *Acronychia pedunculata*

Mulyadi Tanjung<sup>1,\*</sup>, Intan Nurmalasari<sup>1</sup>, Aisyah Kanti Wilujeng<sup>1</sup>, Ratih Dewi Saputri<sup>1</sup>, Fida Rachmadiarti<sup>2</sup>, and Tjitjik Srie Tjahjandarie<sup>1</sup>

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**Abstract** – A new isoprenylated acetophenone, acronyculatin P (**1**) as well as two known compounds, 3',5'-diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**) and 3'-isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**) were isolated from the stem bark of *Acronychia pedunculata* (L.) Miq. The structures were determined by HRESIMS, 1D and 2D NMR. The inhibitory activity of the isoprenylated acetophenone derivatives against murine leukemia P-388 cells showed compound **1** moderate activity with IC<sub>50</sub> 15.42 μM.

**Keywords** – Acronyculatin P, isoprenylated acetophenone, *Acronychia pedunculata*, P-388 cells

### Introduction

*Acronychia pedunculata* is one species belongs to the Rutaceae family found in all of Indonesia. The stem bark have been used in traditional medicine for the treatment of fever, asthma, diarrhea, and rheumatism.<sup>1</sup> According to previous studies, the most common secondary metabolites isolated from *A. pedunculata* are alkaloids,<sup>2</sup> coumarins,<sup>3</sup> and isoprenylated acetophenone derivatives.<sup>4,5</sup> Isoprenylated acetophenone derivatives in the genus *Acronychia* indicate not their value as chemotaxonomic markers of the genus. Isoprenylated acetophenone derivatives were reported to possess cytotoxic,<sup>4</sup> anti-inflammatory,<sup>6</sup> and antioxidant<sup>7</sup> activities. In the present study, a phytochemical investigation is reported of the stem bark of *A. pedunculata* focused on the isolation and structural elucidation of a new isoprenylated acetophenone derivatives, acronyculatin P (**1**) along with two known compounds, 3',5'-diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (**2**) and 3'-isoprenyl-2',4',6'-trihydroxyphenylethanone (**3**). The cytotoxic activity of compounds **1** - **3** against murine leukemia P-388 cells from this plant are also reported.

### Experimental

**General experimental procedures** – Column chromatography and radial chromatography were carried out using silica gel 60 and silica gel 60 PF<sub>254</sub> (Merck, Darmstadt, Germany). UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-VIS spectrophotometer (Kyoto, Japan). IR spectra were recorded in KBr on a One Perkin Elmer instrument (Waltham, MA, USA). NMR spectra were measured on a JEOL JNM-ECA 400 MHz FTNMR spectrophotometer (Tokyo, Japan) in CDCl<sub>3</sub> with TMS as the internal standard. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, [M-H]<sup>-</sup> negative ion mode (Santa Clara, CA, USA).

**Plant materials** – The dried and powdered of stem bark of *A. pedunculata* was collected in July 2017 from Gunung Salak, Bogor, West Java, Indonesia by Mr. Ismail Rachman. The plant material was identified at the Herbarium Bogoriense, Bogor. A voucher specimen (AP 60329) was deposited in Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

**Extraction and isolation** – The stem bark of *A. pedunculata* (1.5 kg) was extracted with methanol at room temperature two times and then extracts were concentrated in vacuo. The methanol extract (350 g) was

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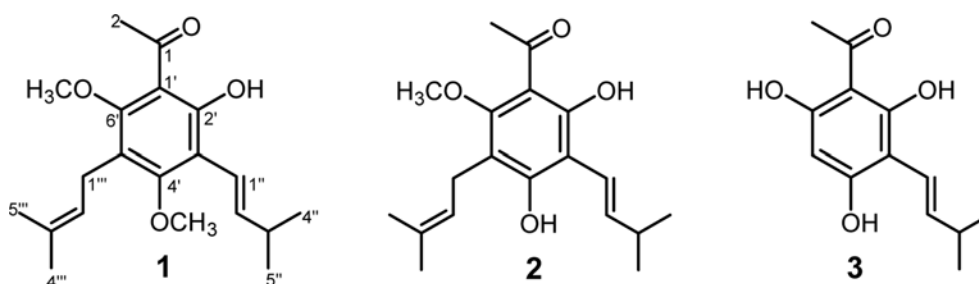


Fig. 1. Isoprenylated acetophenones **1** - **3** isolated from *A. pedunculata*.

suspended in H<sub>2</sub>O and partitioned with *n*-hexane (11.6 g) and ethyl acetate (7.7 g). The *n*-hexane extract (11 g) was further fractionated by column chromatography on silica gel (200 g) eluted with *n*-hexane-ethyl acetate by increasing polarity (from 9:1, 4:1; 7:3, and 1:1) to give three major fractions A-C. Fraction A (0.5 g) was separated by planar radial chromatography eluted with *n*-hexane-CHCl<sub>3</sub> (from 9:1 to 4:1) to produce subfractions A<sub>1</sub>-A<sub>2</sub>. Subfraction A<sub>2</sub> was purified by planar radial chromatography using *n*-hexane-diisopropylether (from 9:1 to 4:1) to yield compound **1** (46 mg). Fraction B (5.3 g) was refractionated using column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 1:1) to produce three subfractions B<sub>1</sub>-B<sub>3</sub>. Subfraction B<sub>2</sub> was purified by planar radial chromatography using *n*-hexane-diisopropylether (from 9:1 to 4:1) to afford **2** (28 mg). The ethyl acetate extract (7.5 g) was fractionated over silica gel column chromatography eluted with *n*-hexane-ethyl acetate (from 9:1, 4:1; and 1:1) to give five major fractions D-H. Fraction E (0.75 g) was purified with *n*-hexane-ethyl acetate (from 4:1, 7:3; and 1:1) to afford **3** (24 mg).

**Acronyculatin P (1)** – Yellowish syrup. UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 252 (4.25), and 283 (4.23). IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3300, 1619, 1587 and 1184. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1. HRESIMS:  $m/z$  [M-H]<sup>-</sup> calcd. for C<sub>20</sub>H<sub>28</sub>O<sub>4</sub> 331.1924, found 331.1909.

**3',5'-Diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone (2)** – Yellowish syrup. UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 224 (4.38), and 290 (4.21). HRESIMS:  $m/z$  [M-H]<sup>-</sup> calcd. for C<sub>19</sub>H<sub>25</sub>O<sub>4</sub> 317.1536, found 317.1532.

**3'-Isoprenyl-2',4',6'-trihydroxyphenylethanone (3)** – Pale yellowish solid, mp. 173 - 175 °C. UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) : 236 (4.32), and 280 (4.20). HRESIMS:  $m/z$  [M-H]<sup>-</sup> calcd. for C<sub>13</sub>H<sub>15</sub>O<sub>4</sub> 235.2569, found 235.2560.

**Cytotoxic activity** – The human tumor cell used in this work was P-388 cells (murine leukemia) and cultured in RPMI 1640 medium. The P-388 cells were seeded into each 96-well cell culture plate at a density of 3 × 10<sup>4</sup> cells/well and incubated at 37 °C for 48 h against murine

leukemia P-388 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay. Compounds **1-3** dissolved in DMSO by variations in concentration of 100; 30; 10; 3; 1; 0.3 and 0.1 µg/mL with triplicate treatment. The number of cells inhibited by each of compounds **1-3** were measured using microplate reader spectrometer at  $\lambda$  540 nm. Artonin E was used as positive control and DMSO 1% was used as negative control.<sup>8-11</sup> The IC<sub>50</sub> values of the compounds were calculated through extrapolation 50% absorption lines to various concentrations using regression analysis.

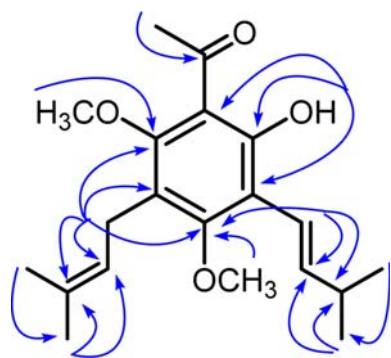
## Result and Discussion

The structures of **1-3** were elucidated by UV, IR, HRESIMS, 1D and 2D NMR spectroscopy. To our knowledge, compound (**1**) is a new compound. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of compounds **2-3** are consistent with published data.<sup>12-13</sup>

Compound (**1**) was isolated as yellowish syrup. The HRESIMS of **1** exhibited a negative molecular ion peak [M-H]<sup>-</sup> at  $m/z$  331.1924 indicating a molecular formula of C<sub>20</sub>H<sub>27</sub>O<sub>4</sub> implying seven degrees of unsaturation. The UV maximum absorption at  $\lambda_{\max}$  252 (4.25), 283 (4.23) nm and IR bands ( $\nu_{\max}$ , cm<sup>-1</sup>) at 3300, 1619, 1587 and 1184<sup>4</sup>. The <sup>1</sup>H NMR spectrum (Table 1) showed an acetyl signal at  $\delta_H$  2.71 (3H, s, H-2), a chelated hydroxyl group at  $\delta_H$  13.43 (1H, s, 2'-OH), and two methoxyl groups at  $\delta_H$  3.71 (6H, s, 4'/6'-OCH<sub>3</sub>). In addition, compound **1** showed 3-methyl-1-butenyl proton signals at  $\delta_H$  6.40 (1H, d,  $J$  = 15.6 Hz, H-1''), 6.55 (1H, dd,  $J$  = 15.6; 7.1 Hz, H-2''), 2.48 (1H, dq,  $J$  = 15.6; 8.2 Hz, H-3''), 1.68 (3H, s, H-4''), 1.10 (6H, d,  $J$  = 6.8 Hz, H-4''/5''), and a 3-methyl-2-butenyl (isoprenyl) proton signals at  $\delta_H$  5.16 (1H, t,  $J$  = 6.6 Hz, H-2'''), 3.29 (2H, d,  $J$  = 6.6 Hz, H-1'''), 1.78 (3H, s, H-4'''), and 1.69 (3H, s, H-5'''). The placement of hydroxyl, methoxyl, 3-methyl-1-butenyl, and 3-methyl-2-butenyl groups of **1** were established by HMQC and HMBC spectra (Fig. 2). The <sup>13</sup>C NMR spectrum of **1**

**Table 1.** NMR Spectroscopic data (400 MHz in CDCl<sub>3</sub>) for acronyculatin P (**1**)

No.C	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	HMBC
1	-	204.7	-
2	2.71 (s, 3H)	31.4	C-1
1'	-	111.9	-
2'	-	161.5	-
3'	-	116.4	-
4'	-	163.5	-
5'	-	120.4	-
6'	-	159.6	-
1''	6.40 (d, 15.6, 1H)	116.7	C-4', C-2'', C-3''
2''	6.55 (dd, 7.1; 15.6, 1H)	143.1	C-1'', C-3'', C-4''/5''
3''	2.48 (dq, 8.2; 15.6, 1H)	32.9	C-1'', C-2'', C-4''/ C-5''
4''	1.10 (d, 6.8, 3H)	22.6	C-2'', C-3'', C-5''
5''	1.10 (d, 6.8, 3H)	22.6	C-2'', C-3'', C-4''
1'''	3.29 (d, 6.6, 2H)	23.2	C-4', C-5', C-6', C-2''', C-3'''
2'''	5.16 (t, 6.6, 1H)	123.8	C-4''', C-5'''
3'''	-	131.6	-
4'''	1.78 (s, 3H)	17.9	C-2''', C-3''', C-5'''
5'''	1.69 (s, 3H)	25.8	C-2''', C-3''', C-4'''
2'-OH	13.43 (s, 1H)	-	C-1', C-2', C-3'
4'-OCH <sub>3</sub>	3.71 (s, 3H)	60.4	C-4'
6'-OCH <sub>3</sub>	3.71 (s, 3H)	63.0	C-6'

**Fig. 2.** Selected HMBC correlations for compound **1**.

revealed the signals for an acetyl group at  $\delta_{\text{C}}$  31.4 and  $\delta_{\text{C}}$  204.7 as well as three oxyaryl carbons at  $\delta_{\text{C}}$  163.5,  $\delta_{\text{C}}$  161.5, and  $\delta_{\text{C}}$  159.6 characteristic for a 2',4',6'-trioxygenated acetophenone derivatives.<sup>5</sup> The HMBC spectrum revealed a cross-peak correlation between  $\delta_{\text{H}}$  2.71 (H-2) and an carbonyl carbon at  $\delta_{\text{C}}$  204.7. The proton signal of a chelated hydroxyl group ( $\delta_{\text{H}}$  13.43, 2'-OH) correlated with three quaternary carbons [ $\delta_{\text{C}}$  111.9 (C-1'); 161.5 (C-2'); 116.9 (C-3')]. The proton signal of methoxyl group at  $\delta_{\text{H}}$  3.71 (4'/6'-OCH<sub>3</sub>) correlated with two oxyaryl carbons [ $\delta_{\text{C}}$  163.5, and  $\delta_{\text{C}}$  159.6] showing the presence of two

methoxyl groups at C-4' and C-6'. Furthermore, the proton signal of methylene of 3-methyl-2-butenyl chain at  $\delta_{\text{H}}$  3.29 has correlation with two oxyaryl carbons [ $\delta_{\text{C}}$  163.5 (C-4'), and  $\delta_{\text{C}}$  159.6 (C-6')], two quaternary carbons [ $\delta_{\text{C}}$  120.4 (C-5'), and  $\delta_{\text{C}}$  131.6 (C-3''')], and a methine carbon at  $\delta_{\text{C}}$  123.8 (C-2''') confirmed that the 3-methyl-2-butenyl chain is located at C-5'. The presence of long-range correlations between the proton signal of a vinylic of 3-methyl-1-butenyl chain at  $\delta_{\text{H}}$  6.40 (H-1'') was correlated to a oxyaryl carbon at  $\delta_{\text{C}}$  163.5 (C-4'), and two methine carbons at  $\delta_{\text{C}}$  143.1 (C-2''), and 32.9 (C-3'') reinforces the location of 3-methyl-1-butenyl chain at C-3'. Therefore, compound **1** was identified as (*E*)-1-(2'-hydroxy-4',6'-dimethoxy-3'-(3''-methylbut-1''-enyl)-5'-(3'''-methylbut-2'''-enyl)phenylethanone and given the trivial name acronyculatin P. Other HMBC correlations consistent with the structure **1** are shown in Table 1 and Fig. 2.

Compounds **1** - **3** were assessed for their cytotoxicity and results are shown in Table 2. The IC<sub>50</sub> values were 15.42, 27.26, and 80.59  $\mu\text{M}$ , respectively (artoinin E as a positive control, IC<sub>50</sub> 3.05  $\mu\text{M}$ ). Compound **1** was more active than others, But, all the compounds were less active when compared with the positive control.



**Table 2.** Cytotoxic activity of compounds **1 - 3** against murine leukemia P-388 cells

Compounds	IC <sub>50</sub> (μM)
Acronyculatin P ( <b>1</b> )	15.42 ± 0.51
3',5'-Diisoprenyl-2',4'-dihydroxy-6'-methoxyphenylethanone ( <b>2</b> )	27.26 ± 1.23
3'-Isoprenyl-2',4',6'-trihydroxyphenylethanone ( <b>3</b> )	80.59 ± 1.67

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